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Regulated acid–base transport in the collecting duct

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Abstract The renal collecting system serves the fine-tuning of renal acid–base secretion. Acid-secretory type-A intercalated cells secrete protons via a lumenally expressed V-type H^+ -ATPase and generate new bicarbonate released by basolateral chloride/bicarbonate exchangers including the AE1 anion exchanger. Efficient proton secretion depends both on the presence of titratable acids (mainly phosphate) and the concomitant secretion of ammonia being titrated to ammonium. Collecting duct ammonium excretion requires the Rhesus protein RhCG as indicated by recent KO studies. Urinary acid secretion by type-A intercalated cells is strongly regulated by various factors among them acid–base status, angiotensin II and aldosterone, and the Calcium-sensing receptor. Moreover, urinary acidification by H^+ -ATPases is modulated indirectly by the activity of the epithelial sodium channel ENaC. Bicarbonate secretion is achieved by non-type-A intercalated cells characterized by the luminal expression of the chloride/bicarbonate exchanger pendrin. Pendrin activity is driven by H^+ -ATPases and may serve both bicarbonate excretion and chloride reabsorption. The activity and expression of

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Keywords Acidosis · Acid–base balance · Ammonium · Bicarbonate transport · pH

Introduction

Extracellular pH and systemic acid–base status are critical for normal organ and cellular function. Deranged acid–base status is associated with higher morbidity and mortality in patients with chronic kidney disease [15]. Extracellular pH affects bone density and stability, and mild chronic metabolic acidosis has been suspected to contribute to osteopenia and osteoporosis [10, 77]. Rickets and osteomalacia are often observed in patients with inborn syndromes of renal tubular acidosis (see below). Extracellular acidosis affects skeletal muscle metabolism and induces a catabolic state [15]. In the setting of chronic kidney disease, metabolic acidosis contributes to peripheral insulin resistance and lower leptin secretion [46, 109, 186]. Thus, extracellular pH has to be tightly kept in the normal physiologic range of pH 7.36–7.44 to maintain normal organ and body function.

Acid–base status is influenced and regulated by the activity of many organs including skeletal muscle (i.e., exercise), intestine (i.e., loss of acid or bicarbonate), bone (i.e., incorporation or release of carbonate and phosphate),

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and by dietary intake or physical activity. Kidney and respiration play a central role in both controlling and maintaining systemic acid–base status by affecting the levels of pCO_2 , bicarbonate, and nonvolatile acids.

The kidney controls and maintains systemic acid–base status by three intricately linked mechanisms: the reabsorption of filtered bicarbonate, the excretion of acids (or, if necessary, of alkali), and the *de novo* generation of ammonium and bicarbonate. The latter process allows the excretion of acids and the replenishing of bicarbonate used to buffer acids. The reabsorption of filtered bicarbonate is mostly achieved by the proximal tubule and to a lesser extent by the thick ascending limb and the distal convoluted tubule (for review, [68]). Urine entering the connecting tubule contains only minute amounts of bicarbonate [68].

Ammoniogenesis occurs only in the proximal tubule; the mechanisms of ammonium excretion will be discussed in more detail below. The ultimate fine-tuning of renal acid or base excretion takes place in the various segments of the collecting system involving various cell types and distinct transport proteins and is subject to tight regulation. The importance of collecting duct acid–base excretion to overall systemic acid–base balance is highlighted by several rare inherited disorders affecting collecting duct acid–base transport proteins or their regulation.

Classic work performed by R.F. Pitts, G. Giebisch, G. Malnic, M.L. Halperin, R. Richterich, R.C. Morris, A. Sebastian, N.E. Madias, H.N. Hulter, W.B. Schwartz, P.R. Steinmetz, R.J. Alpern, Q. Al-Awqati, and many others has elucidated and described the fundamental processes that contribute to urinary acidification using a variety of techniques and animal models. These data are the basis for the more recent molecular approaches dissecting the mechanisms of urinary acidification.

Extensive *in vivo* experiments in various species including dog, guinea pig, rabbit, mouse, rat, and humans indicate that distinct differences exist regarding the basal rates of urinary acidification, the extent of adaptive responses to alkali or acid loading, and the morphological characteristics of the collecting duct (i.e., relative number of different intercalated cell subtypes). Among other reasons, the specific dietary requirements of these species (relative dietary alkali or acid load, electrolyte content) have been discussed as potential explanations. However, it is beyond the scope of this review to discuss these differences.

The purpose of this article is to give a short overview of the mechanisms of acid–base excretion along the collecting system and its regulation on various levels and to discuss briefly dysregulation and inherited disorders of these mechanisms leading to distal renal tubular acidosis (dRTA). This review will discuss mainly more recent data coming from molecular and functional studies in mouse and rats or from genetic studies in humans.

Various segments and cells along the collecting duct contribute to renal acid–base regulation

Cells contributing to final urinary acidification are distributed over several segments along the nephron and collecting system. The first intercalated cells, characterized by the expression of luminal H^+ -ATPases and the anion exchangers AE1 (Anion exchanger 1) or pendrin occur in the late distal convoluted tubule (DCT2) [25, 91, 105, 184]. The subsequent segments of the collecting system, namely the connecting tubule (CNT), the cortical collecting duct (CCD), the outer medullary collecting duct (OMCD), and the initial third of the inner medullary collecting duct (iIMCD) contain various subtypes of intercalated cells. At least two subtypes of intercalated cells can be distinguished based on the expression of specific proteins: type-A intercalated cells and non-type-A intercalated cells (Fig. 1). Type-A intercalated cells are characterized by the presence of luminal H^+ -ATPases and a basolateral anion exchanger, AE1. In contrast, non-type-A intercalated cells express the anion exchanger pendrin on the luminal pole. H^+ -ATPase expression in non-type-A intercalated cells may be luminal, basolateral, or both membranes [91, 184, 202]. However, some authors have further subclassified non-type-A intercalated cells based on subcellular H^+ -ATPase distribution [91, 184]. Non-type-A intercalated cells also express the NHE regulatory factor 1 (NHERF1) [32]. All types of intercalated cells express carbonic anhydrase II and the transcription factor Foxi1 (forkhead box I1) as additional cell-specific markers [27]. Importantly, type-A intercalated cells are dispersed from the late distal convoluted tubule to the initial inner medullary collecting duct. In contrast, non-type-A intercalated cells are mostly expressed in the DCT2 and connecting tubule and less in the cortical collecting duct. Only a few non-type-A intercalated cells are found in the outer stripe of the outer medulla in adult kidney [92, 170, 207]. Pendrin-positive cells are found also in the inner medulla and inner stripe of the outer medulla during nephrogenesis but disappear during the first postnatal days [29, 170].

The classic view states that type-A intercalated cells secrete acid whereas non-type-A intercalated cells are responsible for bicarbonate excretion. This simple classification is challenged by several recent findings. The luminal expression of several subunits of intercalated cell specific H^+ -ATPase has been detected in the principal cells of the connecting tubule (Wagner, Löffing, unpublished observations). Moreover, intercalated cells may serve not only acid–base transport but also the regulation of electrolyte homeostasis. Pendrin function, as discussed below, may contribute importantly to chloride reabsorption along the CNT and CCD. Genetic ablation of the B1 H^+ -ATPase subunit in mice causes a syndrome of massive salt loss

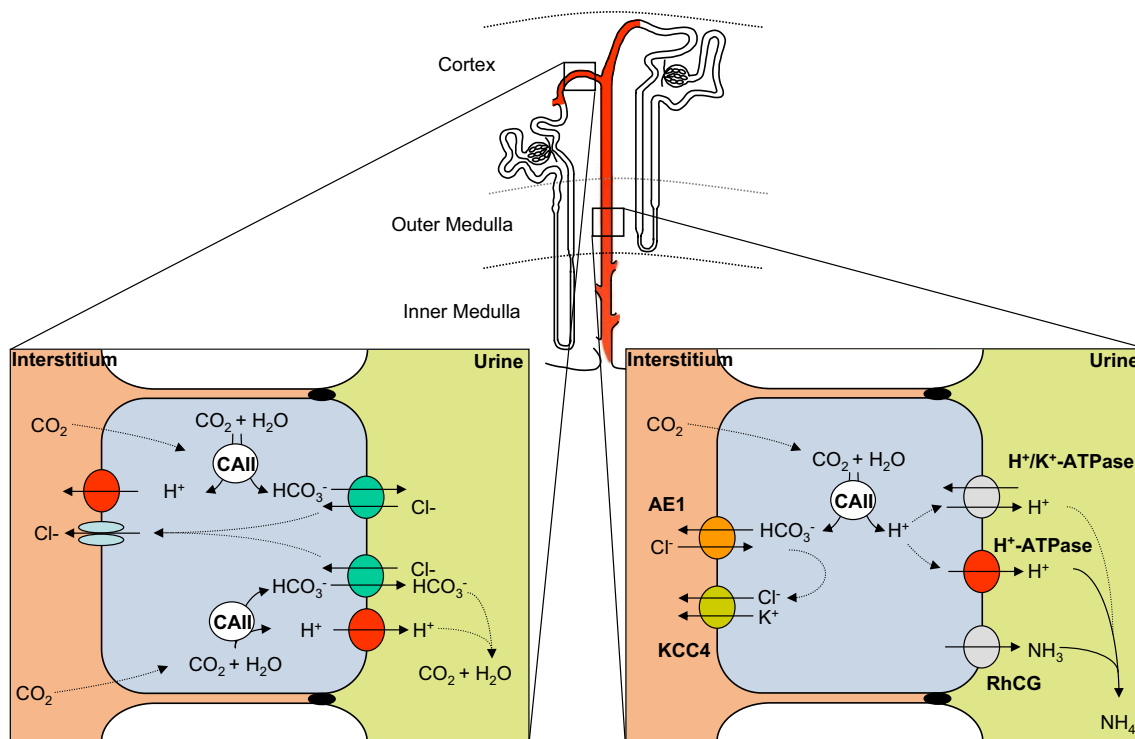


Fig. 1 Two types of intercalated cells. Intercalated cells are expressed from the late distal convoluted tubule to the initial third of the inner medullary collecting duct (red shaded). *Left* cell model of non-type-A intercalated cell. These cells express on the luminal membrane the chloride/bicarbonate exchanger pendrin mediating bicarbonate excretion and chloride absorption. Bicarbonate is produced from CO_2 and H_2O catalyzed by carbonic anhydrase II (CAII). Non-type-A intercalated cells express also V-type H^+ -ATPases which can be found on the basolateral and/or luminal membrane and which may drive pendrin transport activity. Chloride is released across the basolateral membrane through chloride channels that consist of ClC-kb and

Barttin subunits. *Right* cell model of type-A intercalated cell. Bicarbonate and proton generation is catalyzed by CAII providing protons for luminal V-type H^+ -ATPases and bicarbonate for basolateral chloride/bicarbonate exchangers including AE1. Type-A intercalated cells also express basolateral KCC4 KCl-cotransporters that may function in maintaining in low intracellular chloride. Type-A intercalated cells express also on their luminal membrane H^+ / K^+ -ATPases that are not further discussed in this review and serve mostly preservation of potassium during potassium deficiency. Moreover, both type-A and non-type-A intercalated cells participate in ammonium excretion as further detailed in Fig. 3

(Chambrey, Loffing, Wagner, Eladari, unpublished data) which has also been described in patients with classic forms of dRTA [160]. Moreover, expression of flow-activated potassium channels (maxiK) has been described in intercalated cells, thereby contributing to potassium homeostasis [128, 129]. However, it is beyond the scope of this review to discuss the potential role of intercalated cells in electrolyte homeostasis.

The relative contribution of these subsegments to acid–base excretion and final urinary acidification has been difficult to establish. The importance of the segments between the distal tubule and inner medullary collecting duct for urinary acidification has been recognized a long time ago by Ullrich et al. and Gottschalk et al. using microcatheters and microperfusion [63, 191] and the kinetics of acidification were determined under conditions of acidosis and alkalosis by Malnic and Giebisch [61, 199]. These data demonstrated that urinary pH is approximately 0.4 units pH lower than plasma pH in the distal convoluted

tubule and acidifies by as much as 2 units pH reaching values of approximately pH 5.5 at the medullary tip. However, the exact quantitative contribution of single subsegments of the collecting duct system has remained unclear since these segments are not accessible to micro-puncture. Studies in mice lacking either the B1 H^+ -ATPase subunit (ATP6V1B1) or the alpha subunit of the epithelial Na^+ channel (ENaC) have shed some light on the importance of the connecting tubule as a major segment [97]. Application of loop diuretics increases electrogenic urinary acidification, an effect abolished by inhibitors of ENaC function such as amiloride or triamterene [73]. In mice lacking the B1 H^+ -ATPase subunit, specifically expressed in intercalated cells, urinary pH is more alkaline at baseline and does not acidify upon furosemide application. In contrast, in mice that lack αENaC expression in all segments of the collecting duct but with preserved αENaC expression in the connecting tubule, urinary pH is acidified normally after furosemide treatment. These data

indicate that the connecting tubule is sufficient to maintain normal electrogenic urinary acidification [97].

The major transport proteins

H⁺-ATPases

V-type H⁺-ATPases are multisubunit protein complex consisting of at least 14 subunits in humans (for a detailed review on H⁺-ATPase structure, function, and regulation, see recent reviews: [54, 55, 119, 122, 202]). Most subunits occur in different isoforms that may be specific to organs, cell types, or subcellular organelles. In general, H⁺-ATPases are organized in two domains, a cytosolic domain (V₁) binding and hydrolyzing ATP and a membrane bound domain (V₀) mediating proton translocation (Fig. 2). Both domains are connected through a stalk-like structure. Activity of H⁺-ATPases may be regulated by trafficking, domain assembly/disassembly, and changes in the ratio of ATP hydrolysis/H⁺-pumping as well as by other means [55, 122, 202]. H⁺-ATPases couple the hydrolysis of ATP to the

movement of protons across membranes and are found not only in the plasma membrane but are also mostly expressed in many intracellular organelles. Subcellular localization, regulation, and function of different H⁺-ATPase populations may at least in part be regulated by the presence of specific subunit isoforms. The B1, a4, and d2 isoforms have been labeled as intercalated cell specific. However, these isoforms are expressed also in other organs such as epididymis or inner ear and are found also in the thick ascending limb of the loop of Henle (B1) or the proximal tubule (a4) [53, 173]. Localization and expression of the d2 subunit has not been reported in full detail to date [166, 168].

Moreover, staining for several H⁺-ATPase subunits including B1 and a4 has been observed also in cells in the connecting tubule that express ENaC or AQP2 (Aquaporin 2 water channel), typical markers of principal cells. The function of these subunits or full pumps in principal cells has not been investigated in detail. Whether pumps in principal cells of the CNT contribute to urinary acidification is unknown. H⁺-ATPase staining of principal cells is not detected in the CCD and later segments.

Expression of H⁺-ATPases along the nephron during embryonic development in mouse kidney occurs apparently differentially in the different segments of the nephron in a cell-type-specific coordinated manner [79]. Also after birth, H⁺-ATPase expression and abundance increases and reaches adult levels only after about 18–20 days postnatally coinciding with weaning and full urinary acidification [29].

Anion exchangers: AE1 and pendrin

The collecting duct expresses a variety of anion exchangers including members of the SLC4 transporter family ((Anion exchanger isoforms 1–4) AE1, AE2, AE3, AE4, NBCn1 (electroneutral sodium-bicarbonate cotransporter 1)) and the SLC26 transporter family (pendrin, SLC26A7). The role and regulation of AE2, AE3, AE4, NBCn1, and SLC26A7 in the collecting duct are mostly unknown; phenotypes of the respective KO mouse models have not been reported. Thus, this section of the review will focus only on the function and regulation of AE1 and pendrin in the collecting duct.

AE1

AE1 belongs to a subfamily of electroneutral anion exchangers of the SLC4 family of bicarbonate transporters (for review, [7, 38, 138, 144]). The AE1 isoform of the kidney is an N-terminally truncated version of the red blood cell band3/AE1 protein due to alternative splicing of the first exon. Kidney AE1 (kAE1) lacks the first 65 amino acids in humans [8, 96]. AE1 expression is basolateral and its presence characterizes type-A intercalated cells in the

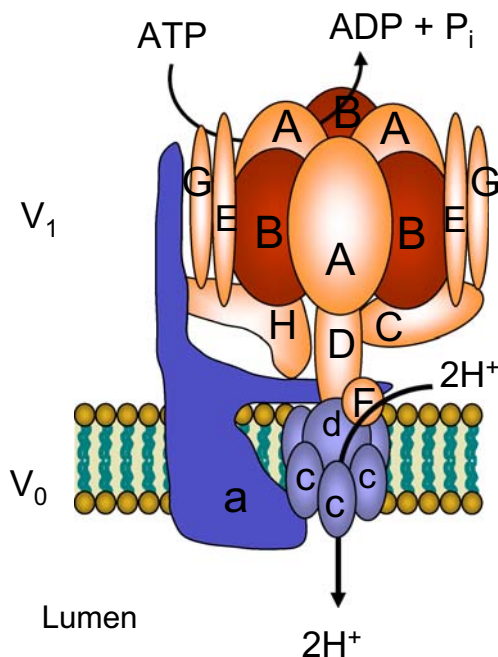


Fig. 2 Model of the structure of vacuolar H⁺-ATPases. H⁺-ATPases are multisubunit membrane-bound enzymes consisting of two major subunits, a cytosolic V₁ domain (shaded in orange-red) and the membrane associated V₀ domain (blue colored). Both domains are connected by a stalk that mediates the energy from ATP hydrolysis to H⁺-transfer. Mutations in the B1 isoform of the B subunit (shaded in red) cause distal renal tubular acidosis with sensorineural deafness. Moreover, mutations have been found in the a2, a3, and a4 isoforms of the a subunit (shaded in dark blue) in patients with cutis laxa (a2), osteopetrosis (a3), or distal renal tubular acidosis (a4). Figure adapted from references [55, 202]

collecting system [9]. AE1 mediates basolateral release of intracellular bicarbonate against extracellular chloride, thereby secreting newly generated bicarbonate into the interstitial space/blood. AE1 may form a transport metabolon together with carbonic anhydrase II which is bound to a C-terminal stretch of amino acids and enhances AE1 transport activity [176]. Similarly, AE1 may also interact with the extracellular carbonic anhydrase isoform IV (CA IV) [175].

The importance of AE1 for normal acid–base status is underlined by the fact that mutations in AE1 cause distal renal tubular acidosis as discussed below. A mouse model lacking AE1 in red blood cells and kidney demonstrated massive hyperchloremic metabolic acidosis [174]. In freshly isolated OMCD type-A intercalated cells, the specific AE1 inhibitor diBA(5)C₄ reduced total chloride/bicarbonate exchange activity only by about 50% and had no effect in OMCDs from AE1 deficient mice. More surprisingly, total chloride/bicarbonate exchange activity was only reduced by 30% in type-A intercalated cells from AE1 KO mice [174]. Thus, type-A intercalated cells express several basolateral chloride/bicarbonate exchangers, but the contribution of AE1 is critical to normal function, which other anion exchanger(s) mediate basolateral chloride/bicarbonate exchange has not been elucidated to date. Due to the lack of specific inhibitors, it has remained difficult to distinguish different anion exchangers.

Little is known about the regulation of AE1 abundance, polarized expression, and activity. Williamson et al. have shown that trafficking of kAE1 may be regulated by the phosphorylation status of two tyrosine residues (Y359 and Y904) which may affect polarization as well as recycling of AE1 [215]. Whether phosphorylation of these residues occurs in vivo and by which stimuli is unknown. Trafficking of AE1 from the Golgi to the plasma membrane may also be regulated through interactions with an integrin-linked kinase [84].

AE1 mRNA and protein expression is increased in rat kidney during metabolic and respiratory acidosis [43, 74, 151]. Interestingly, in mouse kidney, only AE1 protein but not mRNA are enhanced (Mohebbi, Van der Wijst, Perna, Capasso, Wagner, unpublished results). Aldosterone has also been reported to stimulate basolateral anion exchange activity in isolated perfused OMCDs from normal or adrenalectomized rabbit [70]. Presently, it is unknown if this anion exchanger activity reflects AE1 transport activity or alternative transporters. Of note, in mouse kidney, the aldosterone analog deoxycorticosterone acetate (DOCA) increases AE1 mRNA but not protein expression (Mohebbi, Van der Wijst, Perna, Capasso, Wagner, unpublished results).

Pendrin

The anion exchanger pendrin (PDS, SLC26A4) was initially identified as being mutated in patients suffering

from Pendred syndrome (OMIM #274600) characterized by hypothyroidism, goiter, and sensorineural deafness [50, 159]. In the kidney, pendrin is specifically expressed on the luminal membrane of non-type-A intercalated cells [92, 149]. There pendrin may mediate chloride/bicarbonate exchange releasing bicarbonate into urine and reabsorbing chloride. Indeed, both in vivo and in vitro experiments using *Pds* knockout mice indicate that pendrin is critical for bicarbonate secretion during metabolic alkalosis [149]. Accordingly, downregulation of protein expression during NH₄Cl, (NH₄)₂SO₄ or acetazolamide induced metabolic acidosis has been described [57, 67, 203]. During bicarbonate-loading, increased pendrin abundance was observed, whereas potassium depletion caused reduced protein levels, an effect that may further enhance metabolic alkalosis during K⁺ restriction [57, 203].

Moreover, pendrin may also be important for transcellular chloride reabsorption in the connecting tubule and cortical collecting duct. Increased chloride delivery to the distal nephron and collecting duct is associated with reduced pendrin expression levels [139, 192]. Apparently, pendrin expression is sensitive to luminal chloride concentration but less to systemic chloride status. A role of pendrin in collecting duct chloride reabsorption is further supported by the findings that pendrin expression is regulated during chloride depletion [197, 208], *Pds* KO mice are resistant to DOCA and NaCl induced hypertension [196], and angiotensin II stimulates chloride reabsorption in isolated CCDs from wild-type but not from pendrin-deficient mice [132]. However, it is not entirely clear if chloride depletion alone or the accompanying metabolic alkalosis increased pendrin expression. Moreover, the influence of aldosterone or the aldosterone analog DOCA on pendrin expression is controversial. Verlander et al. reported increased pendrin expression in DOCA-treated mice [196], whereas we failed to detect changes in pendrin expression in DOCA-treated mice (Mohebbi, Wagner, unpublished data). Moreover, functional analysis of the pendrin promoter in various cell lines showed in the presence of aldosterone reduced promoter activity when transfected in HEK cells but no effects in other cell lines [1].

Pendrin activity may be controlled on at least four different levels, namely mRNA and protein expression as well as subcellular localization. Enhanced luminal pendrin localization was observed in animals loaded with bicarbonate [203], given DOCA [196], or during chloride depletion [197], whereas several treatments altered total pendrin abundance in the kidney [57, 67, 139, 192, 203]. Studies in various cell lines provided evidence for direct regulatory domains in the promoter region sensitive to intracellular pH and possibly to aldosterone [1]. A fourth and indirect means of regulation may be changes in the number of pendrin expressing non-type-A intercalated cells as observed in

states of chronic metabolic acidosis or altered distal chloride delivery associated with a changed relative abundance of pendrin positive cells [67, 192, 203].

The exact role of pendrin in humans has not been established to date. A recent case report described development of massive hypochloremic metabolic alkalosis in a patient with Pendred syndrome upon treatment with thiazide diuretics [134] which might indicate that pendrin may indeed be necessary to defend against or compensate for metabolic alkalosis as in the pendrin deficient mouse model.

The KCl-cotransporter KCC4

Genetic ablation of the KCl-cotransporter KCC4 was reported by Boettger et al. to cause distal renal tubular acidosis [28]. KCC4 was found to be expressed basolaterally in type-A intercalated cells, Kcc4 deficient mice excreted more alkaline urine and had lower arterial base excess values indicative for renal tubular acidosis. However, it is not clear if this acidosis is of the distal type since KCC4 is also abundantly expressed in the proximal tubule and urinary bicarbonate levels were not reported. Intracellular chloride concentrations were measured in single intercalated cells using energy dispersive X-ray microanalysis and found to be elevated. The authors proposed that KCC4 serves in type-A intercalated cells to release chloride back into interstitium and thereby maintain AE1 activity [28]. However, type-A intercalated cells appear to express also the ClC-Kb/Barttin chloride channel on the same membrane which may serve a similar function. Thus, the role of chloride transporting proteins in the basolateral membrane of (type A) intercalated cells remains to be fully elucidated.

Ammonium excretion: Crucial role of RhCG

The process of renal ammonia/ammonium excretion is complex, involves several nephron segments with distinct mechanisms and is only partly understood (for review, [62, 95, 213, 214]). In the proximal tubule, ammonium is formed from the metabolism of glutamine, a process stimulated by a variety of factors including metabolic acidosis, glucocorticoids, or potassium depletion. Import of glutamine in the proximal tubule cells occurs most likely through the basolateral SNAT3 glutamine transporter regulated by acid–base status and glucocorticoids [65, 83, 115, 123]. The ammoniagenic pathway produces NH_3 and bicarbonate and is also highly regulated during metabolic acidosis [41, 42, 75, 123]. Ammonium is excreted in the proximal tubule either into venous blood or into urine via an apical sodium/proton exchanger (NHE), most likely involving the NHE3 isoform which accepts NH_4^+ instead of H^+

[68]. However, other isoforms, such as NHE8 [64], may also participate as the *Nhe3* deficient mouse model with transgenic overexpression of NHE3 in the intestine shows no major derangement of systemic acid–base status as indicated by normal blood bicarbonate levels [217]. In the thick ascending limb of the loop of Henle, ammonium is actively reabsorbed via luminal $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ (NKCC2) cotransporters where ammonium is accepted instead of potassium. Additional NH_4^+ may enter cells through luminal potassium channels, possibly renal outer medullary K^+ channels. There is also a component of “passive” transport via the paracellular route driven by the lumen-positive potential [62]. Ammonium is released via the basolateral membrane into interstitium by not completely characterized and understood mechanisms. A role for the electroneutral sodium-bicarbonate cotransporter NBCn1 (Slc4a7) has been proposed [76, 126]. Release via isoforms of the KCl cotransporter subfamily might be another possibility; however, the exact localization of the KCC1–4 isoforms along the nephron has not been reported in detail. This step of massive reabsorption by the thick ascending limb epithelium accumulates high concentrations of ammonia/ammonium in the interstitium, thereby providing the driving force for the uptake of interstitial ammonia/ammonium by the adjacent collecting duct cells.

The final step of ammonia/ammonium excretion is mediated by the collecting duct. The major site of ammonia/ammonium excretion is the outer medullary collecting duct. However, during metabolic acidosis, a strong increase in ammonia/ammonium excretion is also found in the connecting tubule, cortical collecting duct, and inner medullary collecting duct [95]. Ammonium accumulates in the medullary interstitium to high concentrations due to the reabsorption in the thick ascending limb. It is thought that this high ammonium concentration provides at least part of the driving force for ammonium excretion into urine. Ammonium secretion results most likely from the trapping of ammonia in the tubular lumen as ammonium after being titrated by protons stemming from active H^+ secretion (possibly driven by V-type H^+ -ATPases). Thus, luminal acidification by the H^+ -ATPases contributes to the driving force for ammonium secretion. In the inner medullary collecting duct, H^+ secretion maintains acidic urinary pH and, thereby, stabilizes NH_3 secretion (as a result of a larger NH_3 gradient between interstitium and acidic urine (low NH_3 but high NH_4^+ concentrations)). Thus, evidence from functional experiments indicates that ammonium secretion along the collecting duct requires a large NH_3 permeability and active H^+ secretion. During metabolic acidosis, ammoniogenesis increases; ammonium reabsorption in the thick ascending limb is stimulated (partly by increasing NKCC2 expression [12]), ammonium accumulation in the medullary interstitium is enhanced, and H^+

secretion along the collecting duct is increased (to a large extent due to increased insertion of functional H^+ -ATPases into the luminal membrane of type-A intercalated cells). Exocytosis of H^+ -ATPase containing vesicles may be directly stimulated by ammonium via a v-SNARE dependent mechanism [56]. Taken together, these factors favor largely increased ammonium excretion along the collecting duct.

Ammonium excretion in the collecting duct requires at least two transport steps, basolateral uptake, and luminal excretion. These mechanisms may be functionally and molecularly distinct. Detailed studies by Susan Wall and colleagues demonstrated several uptake mechanisms for ammonium on the basolateral side, namely $Na^+/K^+/2Cl^-$ cotransport, possibly NKCC1, and Na^+/K^+ -ATPase [204, 206]. In both cases, NH_4^+ can be transported instead of potassium. Little evidence has been obtained for a specific ammonia transport pathway. The role of NKCC1 in basolateral NH_4^+ uptake may be rather small since pharmacological blockade or genetic deletion of NKCC1 has little effect on ammonium excretion [205, 209].

In contrast, the luminal membrane has a high permeability for NH_3 [222]. H^+/K^+ -ATPases are expressed on the luminal membrane and in the case of the so-called colonic isoform, the possibility of active transport of ammonium has been demonstrated [36, 40, 180]. Whether this is relevant in vivo is unknown at present.

The prevailing hypothesis was that ammonium excretion in the collecting duct occurs through nonionic diffusion in the form of NH_3 , subsequent protonation, and trapping of NH_4^+ in the lumen. This process would not require any specific transport proteins and be regulated only by intracellular NH_3 concentrations and luminal acidification.

In 2000, Marini and colleagues provided evidence for a role of Rhesus-like proteins in ammonium/ammonia transport using yeast complementation studies. Three mammalian proteins, RhAG, RhBG, and RhCG were identified [111]. RhBG and RhCG were found to be expressed in mammalian kidney where RhBG is expressed in the basolateral membrane and RhCG on the luminal membrane of all cells along the connecting tubule and intercalated cells only in the cortical and medullary collecting duct [49, 140, 198]. The subcellular localization of RhCG has remained, however, controversial. Eladari et al. described only luminal staining for RhCG in rat kidney [49, 140], whereas the laboratory of D. Weiner has reported both luminal and basolateral staining for RhCG in human [69] and rat kidney [88, 162] and also in mouse kidney [89, 198].

Several studies addressed the possibility that RhBG and RhCG may underlie NH_4^+/NH_3 transport in the collecting duct by studying their transport properties in various cell models [16, 117, 224], their regulation during metabolic acidosis [162, 163], or by generating Rhbg deficient mice [34]. Taken together, these studies indicate that RhBG and

RhCG can mediate transport of NH_4^+ and/or NH_3 but the stoichiometry, the species of transported ions, and transport mode have remained controversial. Additional insights into the transport mechanism and species of transported ions/molecules mediated by Rh proteins came from genetic ablation of Rh proteins in green alga and from crystallization of the bacterial homologue AmtB. Evidence from green alga suggests that the Rh1 protein may be linked to a bidirectional CO_2 gas channel [171]. The crystal structure of the AmtB demonstrates a binding site for NH_4^+ which led to the hypothesis that NH_4^+ is deprotonated to NH_3 which then may permeate the transporter/channel as uncharged gas [85, 94, 223]. At the intracellular face of the protein, NH_3 may be protonated again. Thus, Rh proteins may form part of an ammonia permeable gas channel and may be involved in the transport of NH_4^+ or NH_3 in the kidney collecting duct (Fig. 3).

Two recent studies directly addressed the role of Rhbg and Rhcg in the mammalian kidney performing genetic deletion of these genes. Surprisingly, Rhbg-deficient mice showed no defect in renal ammonium excretion in vivo and ammonium or ammonia permeabilities in the isolated perfused collecting duct showed no difference [34]. In contrast, deletion of Rhcg causes incomplete distal renal tubular acidosis with more alkaline urine, metabolic acidosis, and impaired renal ammonium excretion [26]. Under basal conditions, only a mild reduction in urinary ammonium excretion was found in Rhcg KO mice and no evidence detected for metabolic acidosis. Oral acid challenges caused a much more pronounced metabolic acidosis due to a massive reduction in urinary ammonium excretion whereas titratable acids were normally excreted. The renal defect was further analyzed on the cellular level using in vitro intracellular pH measurements of microperfused CCD and OMCD demonstrating a decrease in the alkalinization rate during luminal application of NH_4Cl suggesting reduced net NH_3 permeability by almost 70%. Similarly, assessment of transepithelial NH_3 permeability in microperfused CCDs showed massively impaired NH_3 fluxes. Thus, Rhcg is required for renal ammonium excretion and may be involved in mediating luminal net NH_3 efflux [26]. This study, hence, established a new paradigm of ammonium transport in the collecting duct requiring the presence of RhCG and indicating that ammonia excretion does not occur by simple nonionic diffusion.

A few studies have also addressed the regulation of RhBG and RhCG during states of increased or altered urinary ammonium excretion. In rodent models of metabolic acidosis, reduced renal mass, or cyclosporine-induced acidosis, no evidence for altered Rhbg abundance or localization was obtained. In contrast, the group of D. Weiner has reported that Rhcg protein abundance is strongly enhanced and that Rhcg staining becomes more

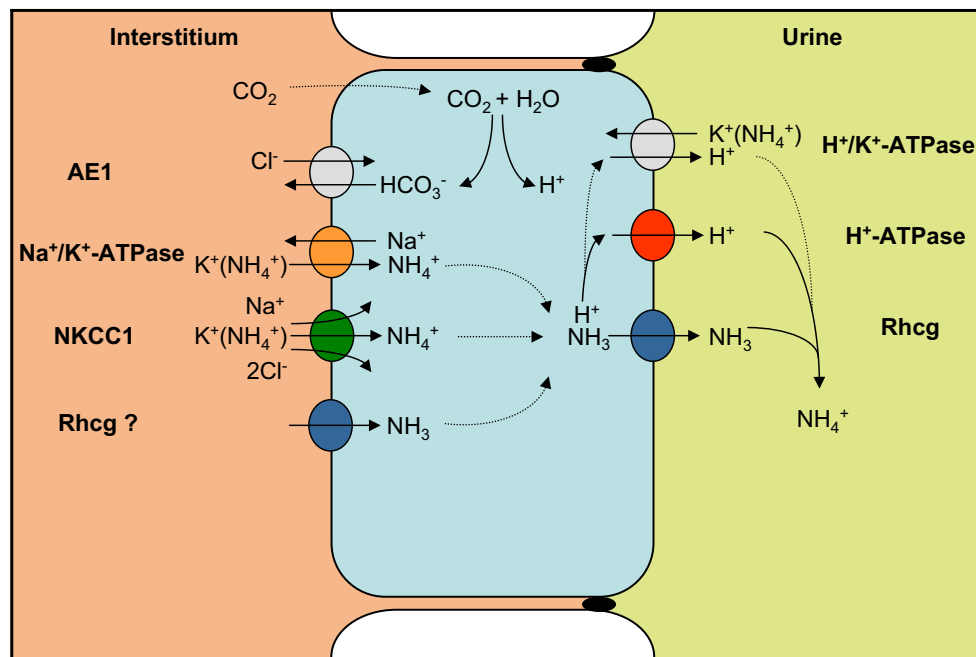


Fig. 3 Ammonium excretion in the collecting duct. Ammonium is excreted into urine by an active, regulated, and at least two-step process. First, ammonium is taken up into collecting duct cells (mostly intercalated cells) from interstitium. This step may be mediated by several transport proteins localized in the basolateral membrane that are able to accept NH_4^+ instead of K^+ ions: the Na^+/K^+ -ATPase and the NKCC1 $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporter. The existence of basolateral

RhCG proteins is controversial. On the luminal membrane, RhCG is expressed and is involved in the net flux of NH_3 . The exact transport mechanism, however, remains to be established. Parallel secretion of protons, mostly by H^+ -ATPases, and to a lesser extent by H^+/K^+ -ATPases acidifies urine and traps NH_4^+ in the lumen, thereby leading to its final excretion

luminal under these circumstances [162, 163]. In contrast, we found in several mouse models of increased ammonium excretion reduced Rhcg mRNA levels (Devuyst, Wagner, unpublished results). Clearly, further work is required to address this discrepancy and to understand acute and chronic regulation of these interesting transport proteins.

Regulation

Acute regulation by hormones and other factors: angiotensin II and aldosterone, CaSR

Acid excretion along the collecting duct is tightly regulated by both systemic as well as local factors. The renin–angiotensin II–aldosterone system (RAAS) appears to be a major stimulator. RAAS activation occurs during metabolic acidosis [13, 66, 80, 153] and blockade of the RAAS impairs renal acid excretion during acidosis [72, 161]. Moreover, deficiency of aldosterone secretion or signaling causes hyperkalemic distal renal tubular acidosis [60, 104, 161]. Similarly, animal studies indicate that angiotensin II and aldosterone are important stimulators of collecting duct acid excretion [19, 100, 101, 110]. On a cellular level, both angiotensin II and aldosterone appear to be strong stimuli for H^+ -ATPase activity in type-A intercalated cells. Type-A

intercalated cells express angiotensin receptors type 1 (AT_1R), where angiotensin II increases intracellular Ca^{2+} and stimulates H^+ -ATPase activity in a protein kinase C dependent manner [133, 148]. Also, aldosterone has direct stimulatory effects on type-A intercalated cell function. Hayes demonstrated that adrenalectomy decreased and aldosterone supplementation restored or even increased luminal H^+ -ATPase activity and basolateral chloride/bicarbonate exchanger activity in the rabbit outer medullary collecting duct [70]. Interestingly, also acute stimulatory aldosterone effects could be observed within a few minutes after application to isolated mouse outer medullary collecting ducts. Aldosterone stimulated H^+ -ATPase activity within minutes, an effect not affected by inhibition of the mineralocorticoid receptor or inhibitors of transcription and translation [216]. The nongenomic effect of aldosterone appears also to be mediated by increased intracellular calcium and protein kinase C (Winter, Velic, Kampik, Wagner, unpublished results).

Local factors that seem to regulate intercalated cell function include extracellular Calcium, CO_2 , and pH. Increased pCO_2 stimulates exocytosis of H^+ -ATPase containing vesicles in the outer medullary collecting duct [156]. Moreover, incubation of collecting ducts in vitro at acidic pH enhances type-A intercalated cell function [190].

How type-A intercalated cells sense extracellular pH or changes in pCO_2 is unknown to date.

Hypercalcemia and subsequent hypercalciuria often causes increased diuresis and stronger urinary acidification. In patients with recurrent calcium containing kidney stones, defective urinary acidification has been detected and is thought to promote crystal formation and stone development [185]. Principal and intercalated cells along the medullary collecting duct express on the luminal membrane the calcium-sensing receptor (CaSR) [152]. Acute activation of the CaSR has been shown to blunt vasopressin stimulated water reabsorption [152]. Renkema et al. could recently demonstrate that the CaSR plays also a major role in urinary acidification and prevention of kidney stones [142]. They used the *Trpv5*^{-/-} hypercalciuric mouse model that presents with massive increased urine flow and acidic urine and showed that generation of double KO mice lacking also the B1 H^+ -ATPase subunit prevented urinary acidification. Hypercalciuric mice with defective urinary acidification developed massive nephrocalcinosis and hydronephrosis and died of renal failure at the age of 8–12 weeks. Moreover, it could be shown that Calcium or the CaSR agonist neomycin stimulated H^+ -ATPase activity in the in vitro microdissected medullary collecting duct from wildtype or *Trpv5*^{-/-} mice but not from B1 KO mice. Thus, activation of the CaSR by high luminal calcium concentrations may trigger a compensatory or preventive process leading to reduced water reabsorption and increased proton secretion thereby reducing the risk of calcium precipitations and kidney-stone formation.

Interestingly, most factors known to stimulate H^+ -ATPase activity in type-A intercalated cells appear to stimulate H^+ -ATPase activity by trafficking of H^+ -ATPases or accessory/stimulatory proteins into the luminal membrane [133, 148, 156, 216]. In the mIMCD3 type-A intercalated cell model, the trafficking of H^+ -ATPase containing vesicles to the luminal membrane involves a protein complex containing Munc-18-2, syntaxin 1-A, SNAP23, and VAMP forming a SNARE complex [5, 17, 18, 102, 121]. Similarly, cleavage of cellubrevin with tetanus toxin prevents stimulation of H^+ -ATPase activity in the outer medullary collecting duct and epididymis [31, 148]. The B1 isoform of the H^+ -ATPase complex appears to play a critical role in the stimulation of H^+ -ATPase activity by trafficking. In the absence of the B1 isoform, type-A intercalated cells express more luminal B2 isoform which may help to sustain basal H^+ -ATPase activity [131]. However, in the absence of a functional B1 isoform, H^+ -ATPases fail to respond to stimulatory factors such as metabolic acidosis, angiotensin II, or CaSR activation [131, 148, 221], thereby explaining at least in part the phenotype of incomplete dRTA in mice lacking the B1 subunit [52].

Regulation through altered protein expression

Acid–base status, electrolytes, as well as some hormones affect abundance of some proteins involved in acid–base transport along the collecting duct. However, it is not clear in all cases if changes in total mRNA or protein abundance are due to true alterations in protein abundance. In some instances, these changes may rather reflect differences in cell number or cannot be clearly distinguished from changes in mRNA or protein expression in total kidney for proteins expressed not exclusively along the collecting duct. Only few reports have been able to overcome the technical difficulties of collecting enough material from dissected and isolated subsegments to examine mRNA or protein levels [35, 123].

Acidosis is associated with increased expression of components of the acid-extruding machinery such as AE1 mRNA and protein as discussed above. In contrast, proteins expressed specifically in non-type-A intercalated cells such as pendrin appear to be downregulated during acidosis, an effect probably due to both reduced number of non-type-A intercalated cells and less transporter/cell as reflected also in greatly diminished immunostaining intensities. Regulation of transcripts has been investigated systematically by serial analysis of gene expression (SAGE) technology using isolated outer medullary collecting ducts from mice subjected to ammonium chloride or potassium depletion-induced urinary acidification. A large number of transcripts encoding for acid–base transporters, regulatory enzymes and kinases, as well as for ion channels was detected [35]. However, it is not clear if these altered transcript levels reflect only changes in transcription or mRNA stability or not also tubular hypertrophy after prolonged treatments. Nevertheless, these data may provide evidence for coordinated regulation of a set of genes in the collecting duct. New technologies such as fluorescence based sorting of specific tubule segments combined with lower requirements for total mRNA for microarray hybridization or more sensitive second-generation sequencing and proteome technologies may offer great avenues for identifying regulated proteins as well as for novel regulators.

Chronic regulation by remodeling

The relative abundance of the different cell types varies along the collecting duct and changes also with electrolyte and acid–base status, a process termed remodeling [3, 14, 158, 203]. During chronic metabolic acidosis, the increase in the relative abundance of type-A intercalated cells has been reported most likely at the expense of non-type-A intercalated cells. Likewise, chronic inhibition of carbonic anhydrase activity or genetic ablation of carbonic anhydrase II causes remodeling of the collecting duct [14, 30].

Apoptotic removal of non-type-A intercalated cells from the medulla during development has been well documented [90, 108, 170]. If this occurs also during adaptation to metabolic acidosis or other electrolyte disturbances has not been reported.

The molecular mechanisms initiating and controlling collecting duct remodeling are not elucidated to date. Reversal of polarity has been discussed based on the observation that H^+ -ATPases change subcellular localization from basolateral to apical membranes during acidosis and that cells with apical bicarbonate secretion acquire basolateral bicarbonate excretion [21, 157, 158, 173]. Cell culture experiments with primary cells from rabbit collecting duct cells suggested that non-type-A intercalated cells may differentiate into type-A intercalated and principal cells [51]. However, uncertainty about the purity of preparations and the lack of good markers for different cell types leave open questions. Interestingly, a protein, hensin (also known as DMBT1 or Muclin), was identified that induced differentiation of cells with characteristics of non-type-A intercalated cells into type-A intercalated like cells in cell culture. The production and secretion of hensin is stimulated during metabolic acidosis and in isolated perfused cortical collecting ducts application of anti-hensin antibodies prevented the reversal of functional polarity suggesting that hensin may play an important role in the acute adaptive remodeling of the collecting duct [2–4, 182, 201]. More recently, three different mouse models deficient for hensin/DMBT1 were reported. Two of these mouse models were apparently viable [44, 143] whereas the third model was lethal at a very early stage [200]. The reason for this discrepancy is unknown at this moment. Polymerization of hensin is required for its effects on epithelia. This complex process may involve interactions with different members of the integrin family and be coordinated by cyclophilin [135, 200].

More recently, the forkhead transcription factor Foxi1 was identified to be highly expressed in intercalated cells. Its genetic ablation causes distal renal tubular acidosis in Foxi1 KO mice due to loss of differentiation of collecting duct cells [27]. It was also shown that the intercalated cell specific AE1, Pendrin, and AE4 transporters may be transcriptionally regulated by Foxi1 [27, 98]. If Foxi1 is regulated during development or by factors that induce collecting duct remodeling is presently unknown. Induction of Foxi1 during nephrogenesis occurs at a time point when first intercalated cells and intercalated cell-specific transport proteins appear further highlighting that Foxi1 plays an important role in intercalated cell differentiation [79].

Proliferation of intercalated cells may significantly contribute to collecting duct remodeling during metabolic acidosis. In mouse kidney, intercalated cells appear to proliferate at a low rate under basal conditions [212]. In

contrast, in rat kidney, we failed to detect evidence for significant basal proliferation of intercalated cells (Bacic, Nowik, Kaissling, Wagner, unpublished data). However, appearance of cells positive for the proliferation markers BrdU, PCNA, or Ki67 occurs as early as 12 h after induction of metabolic acidosis in rats. Interestingly, these proliferating cells appear to be terminally differentiated since cells form part of the tubular lumen and stain positively for the type-A intercalated cell-specific AE1 anion exchanger (Bacic, Nowik, Kaissling, Wagner, unpublished results). Doucet and colleagues had performed SAGE analysis on OMCDs from acidotic mice and detected among other regulated transcripts also GDF15 (growth differentiation factor 15) to be highly upregulated [35]. Using markers of proliferation, these authors report two distinct phases of proliferation in mouse kidney: an early phase around 3 days after induction of metabolic acidosis which may be characterized by axial growth and was abolished in GDF15 deficient mice or when PI3-kinase or mTOR activity were blocked with rapamycin [193]. Consequently, GDF15-deficient mice developed more pronounced metabolic acidosis. The later phase of proliferation occurring 1 week after acidosis induction was not dependent on GDF15 and may be primarily associated with transversal proliferation [193]. Thus, type-A intercalated cell proliferation plays clearly a role in the kidneys adaptation to an acid-load and may consist of distinct phases that are differentially regulated. The fact that the number and relative abundance of different subtypes of intercalated cells may change during metabolic acidosis should be kept in mind in interpreting measurements of total protein abundance of intercalated cell-specific proteins since changes may reflect either altered cell numbers and/or altered protein expression per cell.

Inborn errors of transport: mutations in AE1, B1, a4, and CAII

Rare-inherited familiar forms of dRTA have greatly enhanced our understanding how the collecting duct secretes acid. Syndromes of dRTA are characterized by the inability of the kidney to produce acidic urine ($pH < 5.5$) in the face of metabolic acidosis. dRTA is often associated with disorders of potassium homeostasis (hypokalemic in type I and hyperkalemic in type IV dRTA). Moreover, metabolic acidosis and alkaline urine alone or in combination may promote hypercalciuria and subsequent formation of kidney stones and/or nephrocalcinosis. To date, three different genes have been identified that cause classic type I dRTA when mutated: the SLC4A1 (AE1) anion exchanger [33, 82], ATP6V1B1, and ATP6V0A4 encoding the B1 and a4 subunits of the V-type H^+ -ATPase [81, 169]. Mutations in various components of the aldosterone synthesis or

signaling network cause forms of apparent or pseudohypoadosteronism associated with renal tubular acidosis (so-called type IV dRTA) [104]. Moreover, mutations in carbonic anhydrase II (CAII), expressed in the proximal tubule cells and intercalated cells along the collecting duct, cause a mixed type of dRTA with a combination of proximal and distal features, i.e., bicarbonate wasting and acidification defect [147, 165].

Mutations in either the ATP6V1B1 (B1 subunit) or ATP6V0A4 (a4 subunit) genes cause autosomal recessive forms of distal renal tubular acidosis. B1 and a4 form part of renal H^+ -ATPase pumps [53, 120, 167, 173]. Expression of the B1 isoform is not restricted to intercalated cells but occurs also at lower levels in the thick ascending limb of the loop of Henle. In contrast, the a4 subunit isoform is found in the cells lining the proximal tubule, the thick ascending limb of the loop of Henle, and all subtypes of intercalated cells. Both subunits are also expressed in extrarenal tissues, mainly the epididymis and in cells of the stria vascularis of the inner ear. Differences in male fertility have not been reported to date, whereas patients with ATP6V1B1 mutations suffer from sensorineural deafness early in childhood which is resistant to alkali therapy [81]. Similarly, patients with ATP6V0A4 mutations develop sensorineural deafness which, however, may occur later in life than in ATP6V1B1 patients [177, 194].

Most mutations in the B1 subunit studied to date appear to affect either assembly and/or function of the H^+ -ATPase complex. Experiments in IMCD₃ or HEK cells transfected with various B1 mutants as well as yeast complementation assays with wild-type and B1 mutants found similar defects affecting pump assembly [59, 221]. Interestingly, a B1 variant, considered as rather common polymorphism showed a major defect in the yeast complementation assay suggesting that it may affect pump activity in vivo [59]. Moreover, experiments using mice lacking the B1 subunit demonstrated that the B1 subunit is required for maximal urinary acidification [52]. Under basal conditions, B1 KO mice produced more alkaline urine but had otherwise normal systemic acid–base parameters. However, an oral acid challenge with NH_4Cl caused severe metabolic hyperchloremic acidosis and decompensation [52]. Along the same line, H^+ -ATPase activity in isolated OMCDs was normal under basal conditions but did not increase in OMCDs from acid-loaded B1 KO mice [131] or upon stimulation with angiotensin II [148]. This may be explained by the inability of proton pumps lacking the B1 subunit to traffic to the luminal membrane in response to various stimuli. Indeed, immunohistochemistry showed membrane-associated staining for proton pumps in the OMCD and IMCD of KO mice under basal conditions with enhanced B2 staining [130, 131]. In acid-loaded wild-type mice, proton pump staining in the luminal membrane

of type-A intercalated cells increased whereas no increase was detected in KO mice. Collectively, these data suggest that the B1 subunit appears to confer the ability to H^+ -ATPases to increase their membrane associated activity in intercalated cells in response to external stimuli.

Much less is known about the role of the a4 isoform and the pathomechanisms leading to dRTA in patients. Despite the fact that the a4 subunit is detected along the entire human, rat, and mouse nephron with intense staining in the brush border of the proximal tubule as well as luminal and basolateral localization in all subtypes of intercalated cells [155, 173], no clinical symptoms have been reported from patients indicating reduced function of the proximal tubule. This may be due to the fact that the kidney expresses also the a1, a2, and a3 isoforms of this pump subunit and that expression patterns with a4 widely overlap [155]. The phenotype of an a4 deficient mouse model has not been reported to date. Some mutations in the a4 isoform have been investigated in more detail in vitro. Complementation assays in yeast lacking the homologous Vphlp gene demonstrated defective acidification and growth. One a4 mutant (W520L) demonstrated an interesting phenotype reducing expression of other subunits of the pump suggesting a dominant negative effect [125]. Similarly, the R807Q mutant expresses only low amount of proteins consistent with less stable mutant protein. The a4 isoform apparently interacts with the glycolytic enzyme phosphofructokinase 1 which may link pump function to energy supply [179]. This interaction is disrupted in the G820R a4 mutant leading to decreased pump activity despite an only mildly impaired ability to hydrolyze ATP [178].

Mutations in the AE1 anion exchanger underlie recessive and dominant cases of dRTA and can lead to two distinct phenotypes: red blood cell deformities or distal renal tubular acidosis. Interestingly, in some but not all patients, these two phenotypes do occur simultaneously, but certain mutations are always associated with only one or another phenotype [6, 7]. The literature on AE1 mutations and underlying molecular mechanisms of disease has been summarized in some recent excellent reviews [6, 7, 58, 99, 187, 218]. The most common recessive dRTA-causing mutation, G701D, interacts with the chaperonine glycoporphin which appears to rescue the mutant protein in red blood cells. Glycophorin is absent from intercalated cells possibly explaining the cell-specific phenotype of this particular mutation [183]. A number of other recessive mutations have been described and partially characterized. These mutations are relatively common in Southeast Asia and are often associated with red blood phenotypes [86, 87].

The autosomal recessive pattern of mutations such as G701D, S773P, or the deletion mutant $\Delta 400$ –408, may be explained by the retention of mutant protein intracellularly

whereas enough normal proteins reach the membrane in heterozygous patients [39, 93, 183, 195].

In contrast, dominant dRTA-causing AE1 mutations are rarely associated with red blood cell phenotypes and occur more often in Caucasian patients. Mechanistically, these mutations may affect the polarized localization of AE1 at the basolateral membrane of type-A intercalated cells, remain intracellular, or lose activity [6, 7, 39, 47, 93, 141, 150, 188, 189]. The autosomal dominant pattern of inheritance in certain mutants is possibly due to the fact that the transporter dimerizes or that partial rerouting of mutant AE1 to the luminal membrane of type-A intercalated cells may shunt normal acid secretion.

It should be noted that the impact of AE1 mutations were all studied in various in vitro cell line models relying on stable or transient transfections of mutant AE1. Renal biopsy material from patients with AE1 mutations is rare, and only two cases have been reported in the literature. The S613F mutant is predicted from cell culture models to lead to partially misrouted apical AE1 expression [188]. In contrast, no luminal expression of AE1 was detected in the kidney from a patient carrying the S613F mutation [210]. However, some intracellular AE1 staining was detected; AE1 was absent from the basolateral side. Interestingly, the number of type-A intercalated cells appeared to be greatly reduced in this particular kidney biopsy, and remaining type-A intercalated cells appeared small and abnormal in shape. In the kidney from a patient with the dominant R589H mutation, no AE1 staining was detected in intercalated cells which were reduced in number [164]. Similarly, in mice lacking total AE1 expression, we detected a reduced number of intercalated cells, which might indicate that functional AE1 is required for normal type-A intercalated cell proliferation, differentiation, or survival (unpublished results).

Mutations in CAII are associated with a severe disease characterized by the occurrence of osteopetrosis, (distal) renal tubular acidosis, and cerebral calcifications due to the expression of CAII in all these tissues [147]. In the kidney, CAII is localized in the cytosol of proximal tubular cells as well as in all subtypes of intercalated cells and plays an important role in the intracellular hydration of CO₂ for the generation of bicarbonate and protons [137]. Moreover, CAII is (directly) interacting with several acid–base transporters such as AE1 (see above). Impaired CAII function, thus, leads to reduced generation of transport substrates of bicarbonate and proton translocating pumps and carriers with subsequent loss of bicarbonate reabsorption in the proximal tubule, bicarbonate generation in the collecting duct, and reduced proton secretion by intercalated cells. Studies in rats with chronic pharmacological carbonic anhydrase inhibition with acetazolamide and mice with genetic ablation of carbonic anhydrase revealed both effects

on the remodeling of the collecting duct [14, 30]. Chronic carbonic anhydrase inhibition increased the relative number of type-A intercalated cells at the expense of non-type-A intercalated cells in the CCD, whereas in the OMCD the number of intercalated cells increased and principal cells were reduced [14]. In contrast, genetic deletion of carbonic anhydrase II in mice causes an overall depletion of intercalated cells in all regions of the collecting duct [30]. Thus, chronic impairment of intercalated cell function may impact on differentiation or survival of these cells and lead to replacement by principal cells. Whether this is also the case in human kidney has not been examined to date.

Dent's disease: defective CIC5 chloride/proton exchangers

Dent's disease is primarily a proximal tubule disorder characterized by low-molecular-weight proteinuria that may be associated with hypercalciuria, nephrocalcinosis, and renal failure. It is caused by inactivating mutations of the renal chloride–proton exchanger CIC-5, which colocalizes with the vacuolar H⁺-ATPase in proximal tubule cells and type-A intercalated cells [48, 107, 124]. Investigations of renal biopsies of patients with inactivating mutations of CIC-5 revealed that apical H⁺-ATPase expression was absent in type-A intercalated cells, whereas the polarity of H⁺-ATPase was modified in proximal tubule cells. The significance of these abnormal H⁺-ATPase localizations will need further studies in patients to understand the defect in tubular acidification that is reported in a subset of patients with Dent's disease [116, 154].

Acquired problems of collecting duct acid–base transport

A number of acquired states are associated with dysregulation of collecting duct acid–base transport and can be caused by a variety of diseases such autoimmune disease (Sjögren's disease, autoimmune hypothyroidism), isolated hypothyroidism [114, 127, 219], or hypoaldosteronism. Moreover, a number of drugs may impair the collecting ducts ability to excrete acid or to adapt appropriately to altered systemic acid–base status. These drugs include lithium [11, 22, 113, 118, 146], the immunosuppressants cyclosporine [172] and FK506 [71, 220], amphotericin [112, 145], or toxins such as toluene [24, 181].

Sjögren's disease affects kidney function in about one third of all cases and may cause dRTA [136]. Autoantibodies isolated from patients react with different structures of the kidney including intercalated cells, but the exact antigen(s) have not been further identified. Kidney biopsies have been investigated of few patients and reduced or absent staining for the E and B1 H⁺-ATPase subunit [20, 37, 45, 78, 210] or the AE1 exchanger reported [210]. In a recent series of five patients, we confirmed reduced H⁺-

ATPase expression ($\alpha 4$ and B1 subunits) in intercalated cells, complete absence of AE1 immunoreactivity, and a reduction in the total number of intercalated cells (Mohebbi, Lemaire, Devuyst, Wagner, unpublished results). Thus, Sjögren's disease may cause dRTA due to loss of important collecting duct acid–base transport proteins and less intercalated cells. The order of these events as well as the primary immunologic insult in the kidney need to be further clarified.

The use of the calcineurin inhibitors cyclosporine and FK506 is often associated with dRTA in the setting of patients receiving kidney or other organ transplants [71, 220]. In a rat model, cyclosporine has been shown to cause dRTA [23]. Interestingly, Watanabe et al. demonstrated in the CCD of cyclosporine-treated rats that after an acute acid exposure for 3 h, the adaptive downregulation of bicarbonate secretion by non-type-A intercalated cells was abolished [211]. This effect was secondary to inhibition of the cyclophilin activity by cyclosporine since inhibition of calcineurin alone (by FK506) did not affect the adaptive response in the CCD [211]. Moreover, cyclophilin appears critical for hensin polymerization required to induce terminal differentiation and plasticity of intercalated cells [135].

Application of FK506 to rats did not alter acid–base status or renal acid excretion but transiently caused more pronounced metabolic acidosis upon acid-loading. Detailed analysis of acid–base transport protein expression in the kidney revealed inappropriately high pendrin expression and late reduction in non-type-A intercalated cell numbers during acid-loading (Mohebbi, Wagner, manuscript in revision). These data point again to delayed adaption of the collecting duct due to calcineurin inhibition. However, at least in the case of cyclosporine, dRTA develops independent from calcineurin function suggesting different mechanisms of action of cyclosporine and FK506 [211]. Thus, cyclophilin and hensin may be required for the rapid response of the collecting duct to acid-loads within hours whereas calcineurin may modulate more chronically the expression of pendrin and subsequently the number of non-type-A intercalated cells. A role of calcineurin in the collecting duct is further supported by the fact that calcineurin may be involved in aquaporin-2 water channel regulation and trafficking [103].

Summary and future perspectives

Research over the past 60 years has uncovered major mechanisms of renal acid–base handling, the critical role of the collecting duct in excretion of acids or bicarbonate, identified many molecules involved in these transport processes and their regulation. Transgenic mouse models and rare inherited human diseases have highlighted the

importance of some mechanisms described and have allowed to start dissecting molecular pathways.

Despite this tremendous progress, we still lack insights in major components of the function and regulation of the collecting duct in renal fine-tuning of acid–base homeostasis. The precise mechanism(s) mediating ammonium excretion remained to be uncovered and the role of the Rhesus proteins RhBG and RhCG investigated. Also the role of other transport proteins such as the AE4 anion exchanger, the electroneutral NBCn1 (SLC4A7) transporter, or the K^+/Cl^- cotransporter KCC4 needs to be clarified. Regulation of the H^+ -ATPase, its exact subunit composition in various subdomains of intercalated cells, and its (physical and functional) interaction with other intercalated cell proteins is only poorly understood.

Regulation of collecting duct acid–base handling occurs on various levels ranging from cell proliferation on the one side to acute regulation of transport processes on the other side. We are only starting to understand that and how cell proliferation may contribute to collecting duct acid–base control. Obviously, cell proliferation and differentiation must be controlled and regulated tightly. Moreover, the developmental origin and differentiation of the various cell types making up the collecting duct is only partially elucidated. The role of segment or cell-specific transcription factors such as Foxi1 will be important to understand normal development of the collecting duct as well as regeneration of cells and nephrons. Another major open question is how are remodeling of the collecting duct or transport processes acutely and chronically adapted to the systemic and local acid–base status of the body. Proton activated G protein coupled receptors OGR1 and GPR4 have been identified [106] and are expressed also in the kidney. If these receptors, however, contribute to the control of collecting duct acid–base handling has not been reported. Several hormones have been shown to be increased during metabolic acidosis such as endothelin or the angiotensin II-aldosterone axis. In vivo and in vitro evidence demonstrated their importance. How these and possibly other hormones respond to acid–base status and how they regulate collecting duct function needs to be further examined.

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